Mass spectrometry was used to measure imipramine-induced changes to the lipid profiles of isolated lysosomes. **Results:** There was a clear differentiation between the lipid profiles of lysosomes isolated from control and imipramine-treated macrophages. Significant increases were found in the abundances of specific lipids composed of cholesterol esters, sphingomyelins, and phosphatidylcholines while ceramides and lysophosphatidylcholines were overall decreased. **Conclusions:** These results support the conclusion that imipramine's ability to change lysosomal pH inhibits multiple pH-sensitive enzymes in macrophage lysosomes resulting in increased cholesterol and sphingomyelin levels. Furthermore, more direct targeting of cholesterol accumulation in lysosomes using U18666A to block NPC1 resulted in increased lipid order in isolated lysosome that helps explain the suppression of silica-induced lysosome membrane permeability.

4139 Pulmonary Response to Crystalline Silica and Coal Dust Exposure in Rats

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Background and Purpose: Passage of the Federal Coal Mine Health and Safety Act (Coal Act) in 1969 together with the establishment of the Coal Workers Health Surveillance Program (CWHSP), managed by the National Institute for Occupational Safety and Health (NIOSH) in association with the Mine Safety and Health Administration (MSHA), has greatly reduced the caseload of coal workers pneumoconiosis (CWP) in the U.S. Since the early 2000s, however, the trend has reversed. A significant rise in the incidence of CWP has been reported in the U.S., especially in the central Appalachian states of Virginia, Kentucky, and West Virginia. Yet the etiological factor(s) responsible for the re-emergence of CWP remains elusive. Crystalline silica has been suggested as a potential contributor to the re-emergence of CWP. However, experimental evidence supporting a definite role for crystalline silica in the re-emergence of CWP in the U.S. is lacking. **Methods:** The potential role of crystalline silica in the reemergence of CWP has been investigated by a rat whole-body inhalation-exposure and lung toxicity model. All experiments were done in an Association for Assessment and Accreditation of Laboratory Animal Care International approved animal facility (NIOSH, Morgantown, WV) following a protocol approved by the CDC-Morgantown Animal Care and Use Committee. Aerosols containing crystalline silica (Min-U-Sil 5) or coal dust (Keystone Mineral Black 325BA) were generated by a custom-built automated system. Particle size distribution in the aerosol samples was determined using a micro-orifice uniform deposit impactor (MOUDI). Approximately 3-month-old male Fischer 344 rats (n=6/group) were divided into four exposure groups: 1. Filtered-air control (6 hours/day, 5 days/week during week 1 followed by 6 hours/day, 4 days/week for 3 or 6 months); 2. Min-U-Sil 5 (15 mg/m^3, 6 hours/day, 5 days during week 1 followed by filtered air for 6 hours/day, 4 days/week for 3 or 6 months); 3. Coal dust (filtered air for 6 hours/day, 5 days during week 1 followed by coal dust, 10 mg/m^3, 6 hours/day, 4 days/week for 3 or 6 months); 4. Min-U-Sil 5 + coal dust (Min-U-Sil 15 mg/m^3, 6 hours/day, 5 days during week 1 followed by coal dust, 10 mg/m^3, 6 hours/day, 4 days/week for 3 or 6 months). Rats were euthanized at the end of the 3rd or 6th month, after the first exposure, and bronchoalveolar lavage (BAL) was performed to determine the pulmonary response to exposure(s). **Results:** Mass median aerodynamic diameter of the crystalline silica and coal dust particles in the aerosol samples generated was 1.6 µm [geometric standard deviation (σ_g) 1.6] and 1.36 µm (σ_g 2.3), respectively. At both the time points analyzed, the body weights of the rats belonging to the aerosol exposure groups were less compared to those of the corresponding controls (~5% for coal dust alone and ~10% for crystalline silica followed by coal dust). Exposure to crystalline silica alone resulted in no apparent difference in body weight. Quantification and differential analysis of the bronchoalveolar lavage (BAL) cells revealed significant differences in the aerosol(s)-exposed rats versus time-matched controls. The total number of BAL cells, the number of BAL macrophages (AM), and BAL polymorphonuclear leukocytes (PMN) was higher in rats exposed to the aerosols, either individually or in combination, than the controls. Moreover, at the 3 months interval, these increases were significantly higher (p<0.05) in rats exposed to both crystalline silica and coal dust aerosols than the rats exposed to the individual agents. **Conclusions:** Taken together, the results suggest that exposure to a combination of crystalline silica and coal dust leads to a greater pulmonary response in rats, especially the inflammatory response, compared to exposure to either agent individually. The data underscores the potential role of exposure to crystalline silica in the re-emergence of CWP in the U.S.

4140 Aging alters inflammatory response to crystalline silica in alveolar and bone marrow derived macrophages

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Background and Purpose: Silica and other particles interact with lysosomal membranes and initiate lysosomal membrane permeability (LMP), thereby promoting chronic inflammation through NLRP3 inflammasome activity and cell death. Lysosomal cholesterol has been demonstrated to prevent silica-caused LMP in macrophages, reducing IL-1β release and cell death. Aging is known to increase both cellular and lysosomal cholesterol as well as contribute to chronic inflammation. Here, we examined the inflammatory response of primary alveolar macrophages (AM) as well as bone marrow derived macrophages (BMdM) from aging C57BL/6 mice to determine impacts of aging on macrophage response to inflammation promoting particles. **Methods:** AM and BMdM came from male mice aged 12-weeks and 72-weeks. AM were primed with LPS (20 ng/mL) and treated with crystalline silica (50 µg/mL) for 24 h. Cell death and cytokine release (IL-1β, IL-6, and TNFα) were quantified in supernatants. LMP was assessed using digitonin extraction of silica treated AM to quantify lysosomal enzyme activity in cytosolic extracts. Total lysosomal enzyme was assessed in lysed cells. Lysosomal proteolytic function of AM was assessed. BMdM were treated with crystalline silica and cell death and cytokine release assessed with and without LPS priming. Cellular cholesterol uptake in aging BMdM was assessed. **Results:** AM from aging mice displayed enhanced dose-dependent IL-1β release due to silica, though cell death was comparable to young AM treated with silica. LPS primed aging AM displayed significantly elevated IL-6 levels at baseline compared to younger AM, while IL-1β and TNFα were elevated but not to statistical significance. LPS-primed AM demonstrated a significantly enhanced IL-1β response to silica after 24 h, while silica failed to stimulate increased IL-6 or TNFα in either young or aging AM. Lysosomal enzymes cathepsin B (CTSB) and N-acetyl- β -glucosaminidase (NAG) were measured in cytosolic extracts of AM and aging AM demonstrated significantly less CTSB and NAG following silica treatment for 24 h. Untreated aging AM demonstrated higher cytosolic NAG activity than AM from young mice. Aging AM had higher total NAG activity in lysed cells than young AM. Lysosomal proteolysis probe DQ-Green BSA showed no significant difference in fluorescent intensity between young and aging AM. BMdM from aging mice had significantly less cell death due to silica than young BMdM. Aging BMdM had a stronger IL-1β response to silica without LPS priming but not significantly different IL-1β release due to silica with LPS priming. Aging BMdM had a significantly enhanced IL-6 response to silica with and without LPS priming. Silica failed to stimulate TNFα in either young or aging BMdM. BMdM loaded with TopFluor cholesterol for 24 h showed slightly increased cholesterol. **Conclusions:** Aging is known to contribute to chronic inflammation and can promote a senescence-associated secretory phenotype (SASP) that is associated with high levels of inflammatory cytokines and resistance to apoptosis. In this model of particle-induced inflammation, isolated AM from aging AM demonstrated increased IL-1β, IL-6, and TNFα responses to LPS suggesting a change in age-associated AM response to LPS. Increased lysosomal cholesterol is reported in senescent aging cells and increased lysosomal cholesterol is demonstrated to decrease LMP due to crystalline silica. Here, aging AM demonstrated significantly less LMP due to silica as to be expected with increased lysosomal cholesterol; however, decreased LMP typically corresponds to reduced cell death and IL-1β release, suggesting that additional factors are contributing to the increase in IL-1β release demonstrated in aging AM. Aging cells have been reported to have increased baseline LMP as indicated by NAG activity as well as an increase in lysosomal numbers. The increased cytosolic NAG activity in aging AM suggests an increase in baseline LMP but could also be attributed to increased lysosomal cholesterol, as increased lysosomal cholesterol increases sensitivity to digitonin extraction; however, the increase in total NAG activity in lysed AM observed here suggests an increase in lysosomal number. BMdM from aging mice demonstrated a divergent response to LPS and silica compared to AM. Aging BMdM showed decreased cell death and unchanged IL-1β release with LPS priming and silica treatment, in contrast to the unchanged cell death and dramatically increased IL-1β release in AM. Additionally, aging BMdM demonstrated a significant IL-6 response to silica that was not observed in aging AM. Aging BMdM failed to demonstrate any differences compared to young BMdM in IL-1β, IL-6 or TNFα in response to LPS yet demonstrated increased IL-1β and IL-6 response to silica alone. Taken together, these results suggest aging contributes to an increased inflammatory response to silica in primary AM that is not replicated in aging BMdM. Further work should examine metabolic changes and mitochondrial contributions to silica-induced inflammation in aging AM that could account for the observed increase in IL-1β release.

4141 Host bacterial metabolite spermidine attenuates persistent gastrointestinal toxicity in Gulf War Illness via HMGB1- AhR/NRF-2/HO-1 signaling pathway

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Background and Purpose: Even three decades after the Persian Gulf War, Gulf War (GW) veterans continue to experience persistent gut dysbiosis, which gives rise to gastrointestinal (GI) and neuroinflammation. Following a metabolomics analysis of Gulf War Illness (GWI) stool samples from our lab, we identified a small molecule metabolomic library that identified spermidine as a significant mediator of microbiome metabolism in the host. Spermidine, a naturally occurring polyamine, is known for its anti-inflammatory and anti-aging properties, modulated through the regulation of cell growth, proliferation, and survival. Notably, spermidine is synthesized by intestinal microbes as a bacterial metabolite and has the ability to restore the intestinal gut barrier integrity in conditions like Irritable Bowel Disease and colitis by increasing the production of Kynurenine, an AhR ligand, leading to AhR

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